LIGHT INDUCTION OF NUCLEIC ACID SYNTHESIS IN NORMAL AND CHLOROPLASTMUTANT MAIZE LEAVES

by

I. GYURJÁN, J. N. RAKOVÁN

Department of Evolution and Genetics, Department of Applied Botany and Histogenetics of the Eötvös Loránd University, Budapest

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Introduction

Studies involving lower and higher plants reveal that in the development of chloroplasts a number of nuclear genes are participating, in addition to the information system of the plastid proper (Wettstein and Eriksson 1965). Certain external factors such as light, temperature, and mineral nutrient supply play a similarly important role in this process (Wettstein 1967, Kock and Morison 1958, Homann 1967, Thomson and Weier 1962).

Chloroplast differentiation and its correlation to light at different levels have been studied in biology for quite a long time by now. Analysis of these investigations confirms that light is of great importance in the mechanism of the chloroplast protein synthesis.

Cook and Hunt (1965) demonstrated with synchronized *Euglena* cultures, Chiang and Sueoka (1967) in *Chlamydomonas* tests, that the DNA replication of the chloroplast took place in the light period, whereas that of the nuclear DNA in darkness. The ^{32}P label investigations with tobacco seedlings by Green and Gordon (1966), revealed a rapid appearance of ^{32}P in the chloroplast DNA, with a turnover independent of the nuclear DNA.

The early phases of proplastid-plastid transformation are in close connection with an intensive RNA synthesis. Experiments by Smillie and Krotkov (1960) with *Euglena*, and those of Aoki and Hase (1964) with *Chlorella* show that the green cells contain much more RNA than those developed in the dark. Incorporation of the labelled precursors into the chloroplast RNA is stimulated by light in *Euglena* cells (Zeldin and Schiff 1967) and maize leaves (Bogorad 1967). In etiolated maize leaves, light increases RNA polymerase activity in the chloroplast (Bogorad 1967). Actinomycin D, which blocks the messenger RNA synthesis, inhibits chlorophyll synthesis as well (Pogo and Pogo 1964). This leads to the conclusion that RNA produced by

the light regulation mechanism is an important factor of chloroplast differentiation.

There are still many problems to be solved in connection with the light induction of RNA synthesis. We do not know, for example, the regulator, whereby the light exerts its effect. The quality and quantity of light reguired for induction are not known, either.

The present paper is part of the work aimed at the solution of the said problems, and studies the correlation between the light induction of RNA synthesis on the one hand, and plastid differentiation, on the other, in case of maize leaves of normal and irregular plastid characteristics, respectively.

Materials and methods

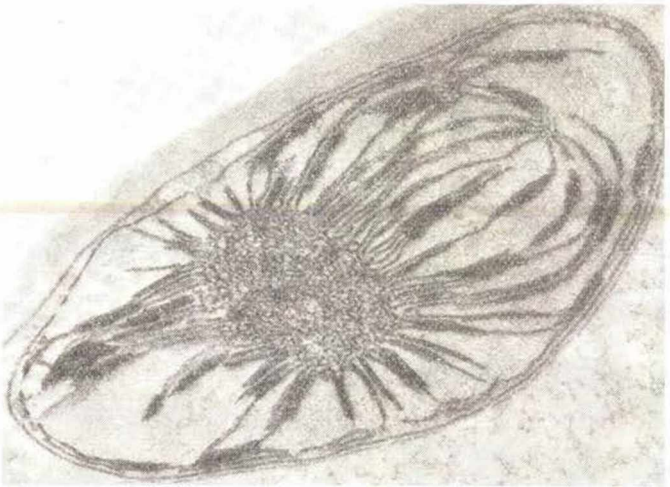
The tests material consisted of normal *Zea mays* L. individuals, their lycopenic and ζ-carotenic mutants (F a l u d i et al. 1960, G y u r j á n et al. 1969). The mutants displayed imperfect chloroplast organization.

Fig. 1a, b, c show electron micrographs of chloroplasts of normal and of mutant leaves illuminated at 100 lux for 12 hours.

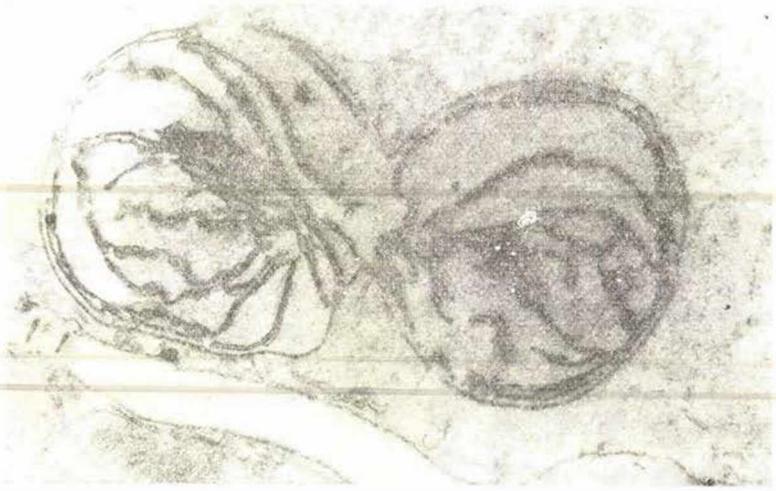
In the chloroplasts of normal leaves (Fig. 1a) we can see the prolamellar body with lamellae are radiating from its and the grana appear.

In the plastids of lycopenic leaves (Fig. 1b) grana formation can be observed, too. These grana (macrograna), however, are longer and thinner than those we have found in the normal leaves.

An irregular structure is characteristic for the chloroplast of ζ-carotenic mutants (Fig. 1c.). In these chloroplasts grana formation is blocked. In most of the chloroplasts we found lamellae with concentric structure and with a lot of vesicles characteristic of highly loosened chloroplasts.



1a



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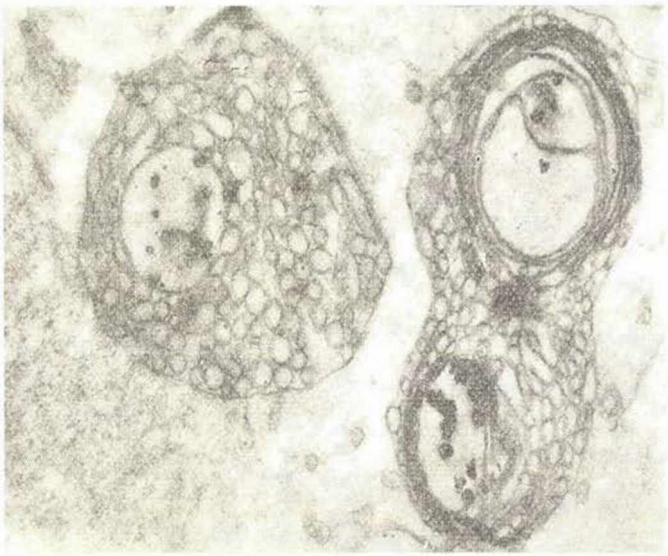

1c

Fig. 1 Chloroplast of normal (a), lycopenic (b) and ζ-carotenic (c) leaves, illuminated with 100 lux light intersity.

In the experiment, the maize grains were kept at a temperature of 26°C in darkness and 100 lux light intensity, respectively, and the raw weight, as well as nucleic-acid content of the leaves were measured by using samples taken every second day.

Nucleic-acid isolation was done by the method of N e c h a y e v a (1966): the leaves were homogenized with 5% $HClO_4$, at 0°C. After centrifuging the homogenate at 10 000x g, the precipitate was repeatedly washed with 96% alcohol, a 1 : 1 ratio mixture of alcohol and ether, and with ether. For nucleic acid hydrolysis, the precipitate was kept in 0.5 n KOH solution at 37°C for 18 hours, then the hydrolysate was cold centrifuged. In order to separate the DNA and RNA fractions, the supernatant was neutralized with $HClO_4$ solution then, after 20 min rest, acidified to a 5% final concentration by adding another quantity of $HClO_4$ solution. Following a short rest period and centrifuging (20 000 x g), the supernatant characterized the RNA while the DNA appeared in the precipitate. DNA was hydrolyzed by boiling with 5% $HClO_4$ at 90°C.

The density of the RNA and DNA fractions was measured, after repeated hydrolysis, at 90°C and 260 mμ by means of a spectrophotometer. The nucleic acid quantity was determined from the optical density of the fractions thus obtained, by using a calibration curve.

Results

The effect of light on the gain in weight and nucleic acid content of normal maize leaves is illustrated in Fig. 2.

The gain in weight of the leaf samples taken daily appeared to deviate from each other with age, when comparing etiolated leaves with those kept in light.

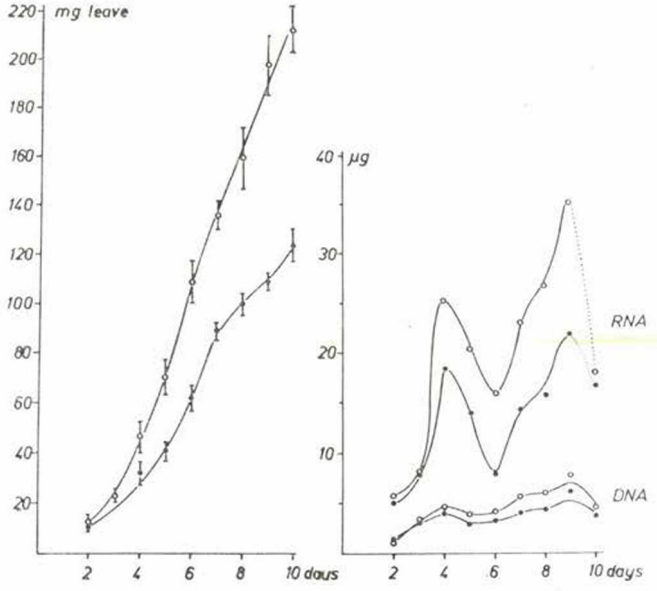

Fig. 2 Variation of the weight and nucleic acid content of normal leaves.
—●—●—●— dark; —○—○—○— Light;

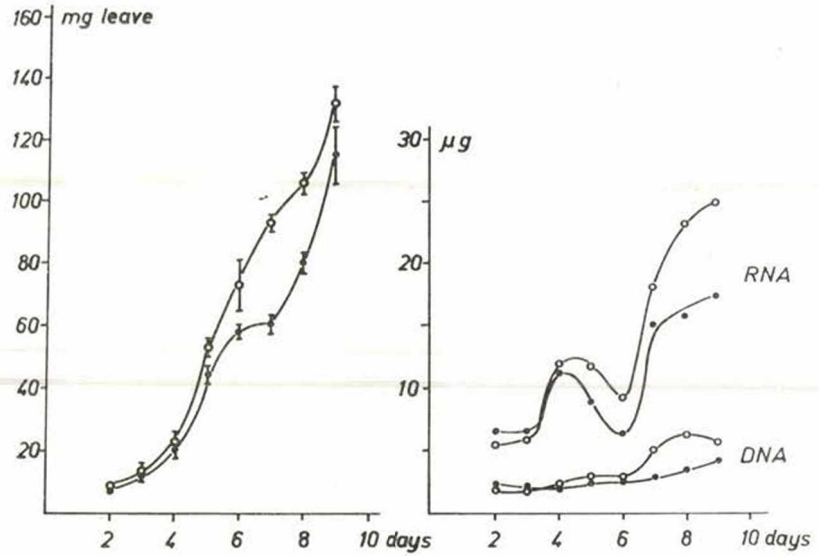

Fig. 3 Weight and nucleic acid content of lycopenic leaves different in age. Symbols: see Fig. 2.

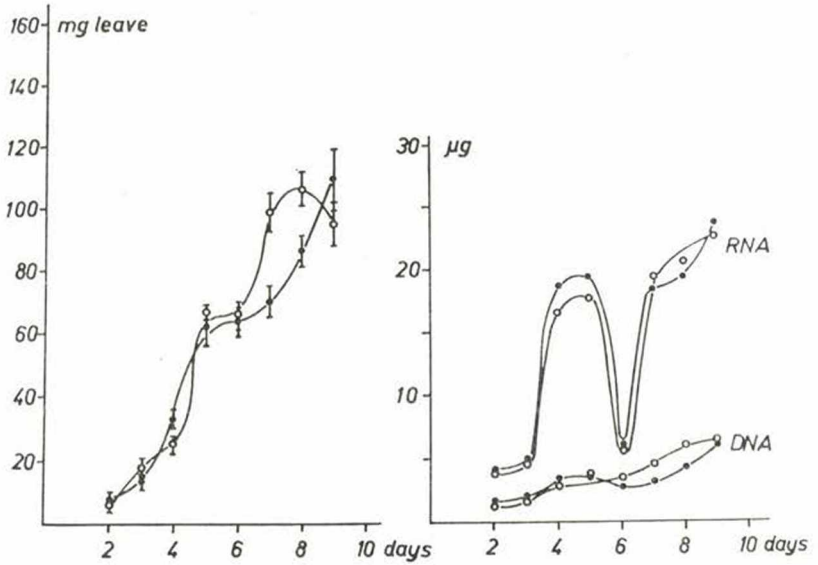

Fig. 4 Development of the leaf weight and nucletic-acid content in ζ-carotenic leaves different in age. Symbols: see Fig. 2.

The RNA content per leaf was always higher in light after the age of 4 days. Interestingly, however, the variation of the RNA content did not closely follow the gain in weight of leaves; in other words, the RNA content appeared to have definite maxima.

In mutants, the weight of the leaves and the RNA content exhibited a tendency similar to that of the normal. The difference is manifested primarily in the fact that in case of lycopenic leaves (Fig. 3) the weight and RNA content of leaves kept in light exceeded, those of the dark variant only to a small extent, whereas in the ζ-carotenic leaves (Fig. 4) this stimulation by light could not be discovered.

In order to know more about light induction, in our experiments the correlation between RNA synthesis and chloroplast differentiation was investigated. For this purpose, etiolated leaves were placed into an aqueous solution of ^{32}P content for 20 hours, after that they were illuminated for 3, 6, 12 and hours, respectively, with a light intensity of 100 lux.

The ^{32}P content of the fractions obtained through the isolation of the nucleic acids was measured, then the light efficiency was expressed as the ratio of inorganic ^{32}P per RNA one values calculated for both the light and dark variants (Table I).

Table I.

The effect of illumination on the RNA synthesis of normal and mutant leaves (light/dark)

Material	Illumination (hours)		
	3	6	24
normal	3,3	4,7	4,6
lycopenic	1,0	1,0	1,2
ζ-carotenic	1,0	1,2	1,2

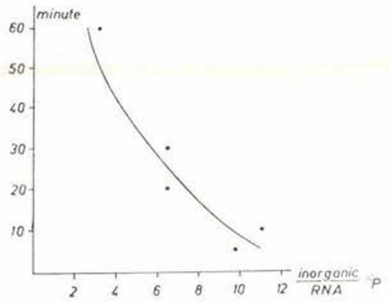

Fig. 5 The effect of preillumination on the incorporation of ^{32}P into the RNA fraction of normal leaves.

In normal leaves, an illumination as short as of 3 hours increased ^{32}P isotope incorporation in to RNA. After an illumination of 6 hours, however, no further increase could be observed. In lycopenic and ζ-carotenic leaves RNA synthesis was not stimulated by light.

Finally, the amount of preillumination required for the initiation of the RNA synthesis was studied. Etiolated normal leaves were illuminated for 10, 20, 30, 40 and 60 min, respectively, after ^{32}P isotope uptake, then kept in dark for 6 hours. Results of this test are shown in Fig. 5. In the present experimental conditions the longer preillumination period was most effective in increasing ^{32}P incorporation to RNA.

Discussion

The experimental data reveal (Figures 2—4) that the variation of the nucleic acid content does not follow the gain in weight of leaves. Similarly, in the experiments conducted by Rhodes and Yemm (1966) in barley seedlings, the variation of the RNA content is characterized by a maximum curve. In our investigations discussed here, the two maxima are probably correlated with the development and ageing of the first and second leaf, respectively. The development of the second leaf actually coincides with the appearance of the second maximum.

Since light does not stimulate ^{32}P inclusion in RNA in mutants, it may be assumed that light induction is connected with the RNA synthesis of the chloroplast proper. However, this assumption is opposed, to the results of Boardman (1966), who found that, upon illumination, the ratio of plastid and cytoplasm ribosomes did not change. In addition, the enzyme proteins of the chloroplast exhibited high activity even in darkness. On the other hand, other investigators (Bogorad 1967, Smillie et al. 1963, Kirk 1964) demonstrated that polymerase activity easy to observe also in the dark, would turn rather intensive in the chloroplast, with the increase of protein synthesis. The ^{32}P investigations conducted by Bogorad (1967) similarly refer to an early indication of plastid RNA.

Experiments on RNA synthesis do not always indicate the employed light intensity. Data in Fig. 5 show that the period of light induction is governed by the intensity of the applied illumination. A determined light energy acts as starter for the RNA synthesis, but thereafter a dark period is required for the development of the suitable RNA level. According to our experiments, this would take about 6 hours.

Summary

Correlation between the light induction of RNA synthesis and chloroplast differentiation was studied in normal and in chloroplast-mutant maize leaves.

The variation of the RNA content of leaves had a definite maximum, not following the tendency of weight gained by the leaves in normal

maize seedlings. In mutant leaves, the tendency of RNA accumulation was similar, but light had a low stimulation efficiency.

The ^{32}P incorporation into RNA was increased by as short as of 3 hours illumination of normal leaves, while in mutant leaves it was practically unchanged under the same experimental conditions. In etiolated normal leaves a preillumination of 60 min, was most effective to increase the ^{32}P incorporation into RNA.

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